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# **Buried wood effects on macronutrient supply and microbial activity and metabolic potential in different oil sands reclamation soils in northern Alberta**

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## **Abstract**

Buried wood is an important yet understudied component of natural and anthropogenic soils. Nutrient immobilization as a response to wood addition during oil sands' reclamation may be a concern since surface wood is salvaged with the soil, thereby becoming buried wood in reclamation cover soils. This project investigated the impact of buried wood on macronutrient supply and microbial communities in different reclamation soils. A 60-day incubation was performed with different rates and types of wood (0%–50%, aspen and pine) and four different soils: fine and coarse forest floor-mineral mix (fFFMM and cFFMM), peat-mineral mix (PMM), and peat. Analysis of macronutrient supply rates using Plant Root Simulator (PRS™) probes and a community-level physiological profiling (CLPP) to assess metabolic potential was performed at the end of the incubation period; microbial activity was measured through soil respiration during the incubation. Responses varied by soil type; however, buried wood caused nitrogen immobilization in three soils due to an increase in the C:N ratio. Soils with lower C:N ratios like fFFMM and PMM were more susceptible to immobilization with a decrease in available nitrogen by up to 95% at a 10% of wood addition. Phosphorus immobilization was observed in cFFMM, and potassium supply increased at 20% of wood and above. Soil microbial activity and metabolic potential increased but no significant changes in the soil profiles were observed. The findings of this study demonstrate that buried wood increases the soil C:N ratio and can potentially cause nitrogen immobilization when added by 10% of volume or more.

**Key words:** buried wood, nitrogen availability, reclamation soils, CLPP, microbial activity

# **Introduction**

Buried wood is defined as deadwood that is buried more than 50% by soil or litter [\(Moroni et al. 2015\)](#page-9-0). In oil sands mining reclamation, wood is buried by a rapid mechanical process that differs from natural forests [\(Moroni et al. 2010\)](#page-9-1). [When starting reclamation operations at a site \(Alberta Envi](#page-9-2)ronment and Water 2012), surface soils are salvaged from upland or lowland ecosystems to be used immediately as cover soil or stored in stockpiles for later use. Before soil salvaging, the merchantable timber is harvested and moved off-site, and the remaining slash and non-merchantable timber is coarsemulched and left on site. Consequently, during soil salvaging operations, this wood is also collected, mixed, and incorporated into the soil, which will be placed as cover soil (20 to 30 cm deep) on a reclamation site. Buried wood represents an input of nutrients and organic matter to the soil, thereby influencing soil nutrient availability, both as a source and as a sink. Buried wood represents a high input of carbon in undisturbed forest soils, approximately 50% of the total wood mass [\(Pettersen 1984;](#page-9-3) [Chandrasekaran et al. 2012\)](#page-9-4), and wood decomposers need nutrients to decompose the material, especially nitrogen since it is necessary for enzymatic activity [\(Robertson and Groffman 2006\)](#page-10-0). Therefore, the soil C:N ratio increases, and as a response the available nitrogen in the soil is consumed by the heterotrophic microbial communities [\(Moritsuka et al. 2004\)](#page-9-5). This process is known as nitrogen immobilization and results in nitrogen not being available for plant uptake [\(Swift 1977;](#page-10-1) [Jansson 1982;](#page-9-6) Jonasson et [al. 1996\). Phosphorus has also been reported to be immobi](#page-9-7)lized with wood additions to the soil [\(Sinsabaugh et al. 1993;](#page-10-2) [Smyth et al. 2016\)](#page-10-3). For that reason, information on thresholds for wood inclusion with soils during salvage can be beneficial to improve land clearing, soil salvage operations, and following cover soil quality. The government of Alberta currently regulates the inclusion of wood chips in soils with the industry directive "Management of Wood Chips on Public Land" [\(Government of Alberta 2009\)](#page-9-8).

There are studies about the effect of wood application on reclamation soils in the oil sands [\(Brown and Naeth 2014;](#page-9-9) [Kwak et al. 2015](#page-9-10)*a*; [Pinno and Gupta 2018\)](#page-10-4), but all of them evaluated coarse woody debris, which is surface wood. The processes and conditions for this surface wood and soil inter-



action are different and may have different outcomes for soil nutrients and microbial communities compared to buried wood. These studies have shown that surface-applied wood increases the soil bacterial biomass and functional group diversity [\(Kwak et al. 2015](#page-9-11)*b*), increases the soil water-holding capacity and vegetation cover [\(Brown and Naeth 2014\)](#page-9-9), supports native plants diversity, and reduces non-native species [\(Pinno and Gupta 2018\)](#page-10-4). The impact of surface-wood application on nutrients is not clear. [Brown and Naeth \(2014\)](#page-9-9) observed a decrease in soil-available nitrate in sites with surface wood, while [Pinno and Gupta \(2018\)](#page-10-4) found that changes in the nutrient supply rates were attributable to the difference in soil types and not to the application of surface wood. Additionally, [Kwak et al. \(2015](#page-9-10)*a*) used trembling aspen wood extract in a laboratory incubation and found that nitrogen availability decreased in both FFMM and PMM as a response to the surface wood extract. Despite these studies, the buried wood component remains unexplored.

The aim of this study was to investigate the impacts of buried wood on the soil macronutrient supplies (N, P, K, Mg, S, and Ca) and on the metabolic potential and activity of the microbial communities in different reclamation soils, and to evaluate whether these impacts vary depending on the soil type and the wood type. Accordingly, we chose to incubate treatments of the most commonly used reclamation soils with different types and amounts of wood to simulate the effect of buried wood while holding a constant temperature and moisture. It is expected that buried wood will have a negative impact on the nitrogen supply rates, since this nutrient is immobilized by the wood-decomposer microorganisms. It is also expected that the responses will vary depending on initial soil C:N ratios.

# <span id="page-2-0"></span>**Materials and methods**

#### Experimental design

A 60-day aerobic incubation was carried out in the lab following a multifactorial design with 4 soil types (coarse forest floor-mineral mix (cFFMM), fine forest floor-mineral mix (fFFMM[\)](#page-3-0), peat-mineral mix (PMM), and peat)  $\times$  [2](#page-3-0) types of wood shavings (pine and aspen)  $\times$  [4](#page-3-0) volumetric percent-ages of wood shavings (0%, 10%, 20%, and 50%[\)](#page-3-0)  $\times$  [3](#page-3-0) replicates  $each = 96$  samples.

## Soils' and wood shavings' characteristics

All soils were collected from different reclamation sites at an oil sands mine in northern Alberta. The fFFMM was collected from the top 3[0 c](#page-3-0)m of a 2-year-old reclamation site. Before placement on the reclamation site, this soil was salvaged from the top forest floor and upper mineral of a Gray luvisol in a "d" ecosite [\(Beckingham and Archibald 1996\)](#page-9-12) with a clay loam texture. The cFFMM was collected from the top 2[0 c](#page-3-0)m (forest floor [+](#page-3-0) [u](#page-3-0)pper mineral), from a "b" ecosite with loamy sand texture, and the peat was collected from the top 0.3 to 1.[3 m](#page-3-0) of an "h" ecosite along with mineral sand. Both of these soils were collected from pre-salvaged sites on the same oil sands mine. The PMM was prepared in the lab using peat and the underlying mineral sand mixed to a volumetric ratio of 60:40 peat to sand; this ratio has been used over the years in reclamation practices when creating PMM [\(Alberta Environment and Water 2012\)](#page-9-2). All the soils were sieved to ensure the removal of large debris such as buried wood, rocks, and coarse roots. Original buried wood was removed from the soils since this study aimed to observe the initial response to wood addition; therefore, wood was removed to stop any wood-related microbial activity during the preincubation period, also to have control on how much wood was added to each treatment. Peat had the highest organic carbon concentration (47.3%) and C:N ratio (21.4); it was also the most acidic soil with a pH of 4.4, while the other soils were more neutral (between 6.3 and 6.9). The fFFMM and PMM had the lowest C:N ratios, and cFFMM was the soil with the lowest electrical conductivity (161.6  $\mu$ [S c](#page-3-0)m<sup>-1</sup>) and the lowest moisture content at field capacity (15.1%). The buried wood that was originally collected with the soils and removed by sieving had the greatest content in the cFFMM (21.2%), while fFFMM and peat had less than 1% in volume [\(Table 1\)](#page-3-0).

The aspen (*Populus tremuloides*) and pine (*Pinus* sp.) woods were kiln-dried shavings with a size range of 1–2.[5 c](#page-3-0)m, commercially used as animal bedding. For aspen, chemical analyses determined a total nitrogen concentration of 0.22% and a total carbon concentration of 48.3%; for pine, a total nitrogen concentration of 0.16% and a total carbon concentration of 48.3% were determined. These two species were selected since they are dominant species in the boreal forest and reclamation areas for this region [\(Beckingham and Archibald 1996\)](#page-9-12). The addition of 10%, 20%, and 50% of buried wood correspond to a field application of 228.6, 457.2, and 1143 $\text{m}^3/\text{ha}$ , respectively, for a cover soil depth of 2[5 c](#page-3-0)m.

#### Incubation

A total of 1L was prepared for each treatment (e.g., pea[t](#page-3-0)  $\times$  [a](#page-3-0)spe[n](#page-3-0)  $\times$  [5](#page-3-0)0[%](#page-3-0) [=](#page-3-0) 50[0 m](#page-3-0)L peat [+](#page-3-0) 500 mL aspen shavings) in re-sealable bags. The treatments were mixed thoroughly by shaking the bags and stirring with a clean spoon to ensure homogeneity throughout the entire content of the bags. Then, for each treatment, three 50[0 m](#page-3-0)L mason jars were filled with 25[0 m](#page-3-0)L of treatment mixture. The content in the jars and in the bags was mixed constantly during the entire process to assure homogeneous treatments for each sample. Aerobicindicator strips were placed inside the jars to monitor the aerobic conditions, and the jars were lightly sealed with a loose lid to ensure air circulation.

The remaining treatment mixtures were used to determine field capacity [\(Cassel and Nielsen 1986\)](#page-9-13) following the same experimental design (triplicates). Saturated samples with ultrapure water were put into a [5 B](#page-3-0)ar Ceramic Plate Extractor (Soilmoisture Equipment Corp., Santa Barbara, CA) under a constant pressure of 0.[3 B](#page-3-0)ar for 2[4 h](#page-3-0) to reach field capacity. Afterwards, the samples were weighed, oven-dried for 2[4 h](#page-3-0) at 1[0](#page-3-0)0 °C, and weighed again; the moisture content at field capacity was calculated using the wet and dry weights of the samples. A moisture of 70% of field capacity was maintained during the entire incubation period. The final weight of all the samples was recorded. The incubation was conducted at

Soil type	$TN(w/w\%)$	TOC (w/w%)	$C/N$ ratio	рH	$EC$ ( $\mu$ S/cm)	$MC$ at FC $(\%)$	$BW (vol\%)$	
<b>CFFMM</b>	0.20b(0.14)	4.27c(0.40)	21.35	6.32b(0.11)	161.60d (0.43)	15.11c (0.88)	21.24	
<b>fFFMM</b>	0.19b(0.14)	3.55c(0.40)	18.68	6.54ab(0.49)	641.0c (0.0)	26.91b (0.32)	0.90	
Peat	2.21a(0.14)	47.25a (0.40)	21.38	4.42c(0.014)	1221a (2.16)	194.38a (1.64)	0.026	
<b>PMM</b>	0.04c(0.14)	8.92b(0.40)	18.20	6.84a(0.024)	764.67b (1.25)	30.47b (8.89)		

<span id="page-3-0"></span>**Table 1.** Physical and chemical characteristics of the soils.

**Note:** Values are mean, and standard errors are in brackets. Letters indicate similarities among soils (Fisher's LSD test, *p* < 0.05). TN, total nitrogen; TOC, total organic carbon; EC, electrical conductivity; MC at FC, moisture content at field capacity; BW, original buried wood content; cFFMM, coarse forest floor-mineral mix; fFFMM, fine forest floor-mineral mix; PMM, peat-mineral mix.

a constant temperature of 2[5](#page-3-0) ◦C to promote microbial activity [\(Pietikäinen et al. 2005;](#page-10-5) [Bárcenas-Moreno et al. 2009\)](#page-9-14) for a pre-incubation period of [2 w](#page-3-0)eeks to first stabilize the microbial activity to the incubation conditions and then the incubation continued for 60 more days. The samples were aired out every two to three days for 30–4[5 m](#page-3-0)in to maintain an aerobic condition and then weighed and rewetted as needed to maintain the 70% of field capacity.

#### Soil analysis

Subsamples were taken from all the samples for soil analysis. For pH and electrical conductivity, 1[0 g](#page-3-0) sieved and airdried (2[4 h](#page-3-0)) subsamples were prepared following the Kalra and Maynard method [\(Kalra and Maynard 1991\)](#page-9-15) with deionized water, and a SevenEasy pH meter and a FiveEasy conductivity meter (© Mettler Toledo) were used. Soil nutrient supply rates were determined using Plant Root Simulator probes (PRS™ probes, Western Ag Innovations, Inc., Saskatoon, SK). A pair of probes was incubated with 10[0 m](#page-3-0)L of each subsample at 70% of field capacity in re-sealable bags for [7 d](#page-3-0)ays at  $25^{\circ}$  $25^{\circ}$  $25^{\circ}$ C under aerobic conditions, the bags were partially opened every two to three days to maintain aerobic conditions and samples were rewetted as needed to maintain the 70% of field capacity. The probes were inserted vertically in the soil and stored vertically in the incubator. After incubation, the probes were rinsed thoroughly with ultrapure water and sent to Western Ag Innovations for analysis. Each pair of probes (sample) was eluted with 17.[5 m](#page-3-0)L of  $0.5 \text{ mol L}^{-1}$  $0.5 \text{ mol L}^{-1}$  $0.5 \text{ mol L}^{-1}$  HCl for [1 h](#page-3-0). The inorganic nitrogen (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) in the eluant was determined by flow injection analysis (Skalar San++ Analyzer, Skalar Inc., Netherlands), and the rest of nutrients in the eluant (P, K, Ca, Mg, S, Fe, B, Pb, Mn, Cu, Zn, Al, and Cd) were determined by inductively couple plasma (ICP) spectrometry (Optima ICP-OES 8300, PerkinElmer Inc., USA) [\(Western AG Innovations Inc. 2010\)](#page-10-6). The minimum detection limit (MDL) for the probes is  $2 \mu$  $2 \mu$ g. For this study, only macronutrients were considered (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>–</sup>, P, K, Ca, Mg, and S).

To assess the metabolic potential of the microbial communities, a community-level physiological profiling (CLPP) was performed by sieving 6[0 g](#page-3-0) of each subsample and loading individual deep-well plates with 15 different substrates (grouped as amino acids, carboxylic acids, and carbohydrates) and deionized water as a control. Each deep-well plate was clamped to an indicator plate with Cresol red as a respiration indicator [\(Baratella and Pinzari 2019\)](#page-9-16) and incubated for [6 h](#page-3-0) at 2[5](#page-3-0)  $\degree$ C. The indicator plates were read before and after

the incubation period (t0 and t6) using a Synergy HTX multimode microplate reader (BioTek Instruments Inc., Winooski, VT, USA). The aim of this analysis was to identify changes in colour as a response to metabolic activity from the consumption of the substrates; therefore, readings indicated  $CO<sub>2</sub>$  production rates ( $\mu$ [g C](#page-3-0)O<sub>2</sub>-[C g s](#page-3-0)oil<sup>-1</sup> [h](#page-3-0)<sup>-1</sup>). Data were transformed [following the MicroResp™ Technical Manual \(Campbell et](#page-9-17) al. 2015). Soil respiration was measured at day[s 0](#page-3-0), 3, 7, 15, 30, 45, and 60 of the incubation period. The mason jars were connected to a multiplexed flask system consisting of a Multiplexer box LI-8150 and an LI-8100 Automated Soil  $CO<sub>2</sub>$  Flux System (LI-COR Biosciences, Lincoln, NE, USA). This system pumped external air into the jars, and the change in the  $CO<sub>2</sub>$  molar fraction was measured during an observation period of [3 m](#page-3-0)in. Data were extracted using the Soil-FluxPro™ Data Analysis Software (LI-COR Biosciences, Lincoln, NE, USA), which used the change in  $CO<sub>2</sub>$  concentration over time (d*C*/d*t*) and the conditions of volume, pressure, and temperature to calculate a  $CO<sub>2</sub>$  flux. The  $CO<sub>2</sub>$ fluxes at day[s 1](#page-3-0)5, 30, 45, and 60 were used to calculate the mean soil respiration, used to represent the soil microbial activity.

#### Statistical analysis

All data analysis was done using R software (version R.3.1.1, R Core Team 2019) unless otherwise indicated. All data were tested for normality and homogeneity of variances with qqplots, fitted vs. residual plots, and Shapiro–Wilk tests in the case of non-normal data, but with homogeneity of variances, log transformations were applied. To test the effects of soil type, wood type, and wood amount on the macronutrient supply rates, the microbial metabolic potential (CLPP), and on the microbial activity (mean soil respiration), three-way Analysis of variances (significance level of 0.05) were performed followed by a Fisher's LSD test as a post hoc test to identify differences and similarities among treatments. Wood type (aspen and pine shavings) was not a significant factor  $(p > 0.05)$  for any response variable and therefore was excluded from further results. A non-metric multidimensional scaling (NMDS) was carried out using PC-ORD (Wild Blueberry Media LLC, Oregon, US), followed by a multiple response permutational procedure (MRPP). For this multivariate analysis, the data were organized in two matrices, one with the respiration rates for each substrate and a second matrix with the respiration rates grouped by carbon source (amino acids, carbohydrates, and carboxylic acids), along with data on pH, EC, and the nutrient supply rates.

**Fig. 1.** Soil inorganic nitrogen supply rates (μg 10 cm<sup>−2</sup> 7 days<sup>−1</sup>) for (a) total inorganic nitrogen (TIN), (b) nitrate (NO<sub>3</sub>−), and (*c*) ammonium (NH4 <sup>+</sup>) in different reclamation soils after a 60-day incubation with different buried wood additions (0%, 10%, 20%, and 50% of volume). FFMM, forest floor-mineral mix; PMM, peat-mineral mix.

<span id="page-4-0"></span>

# **Results**

#### Nutrient supply rates

For all nutrients and treatments, soil type had the largest impact (*[p](#page-3-0)* [< 0](#page-3-0).05), followed by wood amount (*[p](#page-3-0)* [< 0](#page-3-0).05). Among soils, as buried wood addition increased, the total inorganic nitrogen (TIN; [Fig. 1\)](#page-4-0) supply rates decreased gradually until reaching the MDL for the PRS probes at the highest 50% wood application rate. The fFFMM control (0% of buried wood) had the highest TIN supply, but this soil also had the greatest response to buried wood addition with a decrease by up to 95% for the smallest wood addition of 10%. PMM had the next highest TIN supply, a decrease of 40% was observed for a 10% buried wood addition, and an addition of 50% of wood caused the TIN supply rates to decrease by 98%. Peat only had a response at a 20% buried wood addition and above, with a decrease of less than 40%. cFFMM had the lowest TIN supply rate

in the control, and there was no significant response to wood addition  $(p=0.74)$  $(p=0.74)$  $(p=0.74)$  $(p=0.74)$  $(p=0.74)$ . Phosphorus (P; [Fig. 2\)](#page-5-0) supply rates were the highest in the cFFMM control and decreased by up to 35% as the buried wood increased  $(p < 0.05)$  $(p < 0.05)$  $(p < 0.05)$ . Other soils had P supply rates equal or less than the MDL across all treatments and had no significant responses to buried wood (*[p](#page-3-0)* [>](#page-3-0) 0.05). Potassium (K) supply rates had a different response since there was an increase with the buried wood additions and reached the maximum supply at 50% of buried wood (*[p](#page-3-0)* [< 0](#page-3-0).05), increasing by up to 10 times the supply rates in the controls; fFFMM had no significant response for K  $(p > 0.05)$  $(p > 0.05)$  $(p > 0.05)$  $(p > 0.05)$  $(p > 0.05)$ . Calcium supply rates had no response to buried wood in peat and fFFMM ( $p > 0.05$  $p > 0.05$  $p > 0.05$  $p > 0.05$  $p > 0.05$ ); cFFMM and PMM had decreases by up to 45% at additions of 20% and above (*[p](#page-3-0)* [< 0](#page-3-0).05). Magnesium supply rates decreased gradually with buried wood addition in the cFFMM (*p* [< 0](#page-3-0).05), and [p](#page-3-0)eat had no significant response  $(p > 0.05)$  $(p > 0.05)$  $(p > 0.05)$ . Sulphur supply rates decreased gradually with buried wood additions in

**Fig. 2.** Soil macronutrient supply rates (μ[g 10 c](#page-3-0)m−<sup>2</sup> [7 d](#page-3-0)ays−1) for phosphorus (P), potassium (K), magnesium (Mg), sulphur (S), and calcium (Ca) in different reclamation soils after a 60-day incubation with different buried wood additions (0%, 10%, 20%, and 50% of volume). FFMM, forest floor-mineral mix; PMM, peat-mineral mix.

<span id="page-5-0"></span>

peat and cFFMM (p < 0.05). No clear responses were observed in the magnesium and sulphur supply rates in the fFFMM and PMM.

#### Microbial activity and metabolic potential

Buried wood addition increased the metabolic potential of carbon consumption in all the soils except cFFMM ( $p > 0.05$ ) [\(Fig. 3](#page-6-0)*a*). The fFFMM and PMM reported the greatest increase in metabolic potential, with the highest rates of carbon con-sumption, 51 and 8[2](#page-3-0)  $\mu$ [g C](#page-3-0)O<sub>2</sub>-[C g s](#page-3-0)oil<sup>-1</sup> [h](#page-3-0)<sup>-1</sup>, respectively. Peat and cFFMM had lower metabolic potential, with carbon consumption rates 10 times lower. Amino acids and carboxylic acids were the most consumed carbon sources across all treatments [\(Fig. 3](#page-6-0)*b*), accounting for 85% of the carbon consumption in fFFMM, 94% in PMM, and 71% in cFFMM. Contrastingly, in peat, carbohydrates were the most consumed, corresponding to 40% of the overall carbon consumption. Buried wood increased the amino acid and carboxylic acid consumption; in fFFMM, the metabolic potential for these two carbon sources doubled at 50% of buried wood. In PMM, amino acid consumption increased after a 20% addition, and the carboxylic acid consumption was 6.5 times higher than the control at amounts of 10% and 50% of buried wood. In peat, the metabolic potential for consumption of all three carbon **Fig. 3.** (*a*) Metabolic potential in terms of carbon consumption rates ( $\mu$ [g C](#page-3-0)O<sub>2</sub>-[C g s](#page-3-0)oil<sup>-1</sup> [h](#page-3-0)<sup>-1</sup>) for different carbon substrates (amino acids, carboxylic acids, and carbohydrates) as a response to buried wood addition after a 60-day incubation. (*b*) Overall proportion of the different substrates' consumption rate in the soils. FFMM, forest floor-mineral mix; PMM, peat-mineral mix.

<span id="page-6-0"></span>

**Fig. 4.** Microbial activity in terms of mean soil respiration  $(CO<sub>2</sub> flux)$  with different amounts of buried wood during a 60-day incubation. Values are based on the measurements of days 15, 30, 45, and 60. FFMM, forest floor-mineral mix; PMM, peat-mineral mix.

<span id="page-6-1"></span>

sources increased gradually and was at least 2 times higher at 50% of buried wood. No clear pattern was observed in the cFFMM.

Microbial activity [\(Fig. 4\)](#page-6-1) was the highest in fFFMM across all treatments (*p* < 0.05), followed by peat, cFFMM, and PMM. In all soils, microbial activity increased with buried wood (*p* < 0.05); fFFMM and PMM had the highest activity at 20% of buried wood with values 2 times and 6.5 times higher than in the controls, respectively. Peat had no significant response until 50% of buried wood, and in cFFMM, microbial activity increased gradually with wood addition and reached a microbial activity at least 4 times higher than the control.

When compiling the microbial metabolic potential and microbial activity data (CLPP and mean soil respiration, respectively), pH, EC, and the macronutrient supply rates in an NMDS ordination, it was observed that the variation among soils was significantly greater than the variation within the soils due to buried wood [\(Fig. 5\)](#page-7-0). An MRPP supported that all the soils had a significantly different microbial metabolic potential and macronutrient supplies (*p* < 0.05) regardless of the amount of buried wood.

## **Discussion**

Buried wood impacted soil macronutrients and microbial communities, most significantly in fFFMM and PMM. Soil TIN was the most impacted nutrient by buried wood. Microbial activity and metabolic potential increased significantly as a response to buried wood addition. The differences in microbial activity and metabolic potential were greater among soil types than among buried wood amounts, indicating that although buried wood had some significant effects within each soil, soil type was the main factor driving the different responses in the microbial communities. There was no significant difference in macronutrient supply and microbial communities' responses between the aspen and pine wood addition, likely because of the disturbed lignin barrier in the wood shavings resulting from the physical disturbance that occurs when the wood is processed into shavings. This disrupts the lignin walls and exposes the cellulose and [hemicellulose to microbial attack and decomposition \(Kirk](#page-9-18) and Cowling 1984; [Ulyshen and Šobotník 2018\)](#page-10-7). Thus, there was no difference in resistance to breakdown between aspen and pine wood, resulting in similar responses. Another possible explanation is the similar C:N ratio of both wood types (see in [Materials and methods\)](#page-2-0). However, the wood C:N ratio was measured for material characterization and was not part of the experimental design (not enough replicates); therefore, no statistical analysis could be performed with this data.

**Fig. 5.** Non-metric multidimensional scaling (NMDS) ordination of the metabolic potential of microbial communities using community-level physiological profiling in fFFMM, cFFMM, PMM, and peat with different buried wood amounts (stres[s](#page-6-1) = [6](#page-6-1).23%) in response to carbon substrates (amino acids, carboxylic acids, and carbohydrates, and water as control), soil pH and EC, and soil nutrient supply rates. fFFMM, fine forest floor-mineral mix; cFFM, coarse forest floor-mineral mix; PMM: peat-mineral mix.

<span id="page-7-0"></span>

Axis 1 (86.7%)

The observed nitrogen immobilization is similar to previous research that found significant decreases in nitrogen availability as a response to wood addition and increases in [the soil C:N ratio \(](#page-9-19)[Moritsuka et al. 2004](#page-9-5)[;](#page-9-19) Palviainen et al. 2010; [Mukhortova 2012;](#page-9-20) [Truong and Marschner 2018\)](#page-10-8). Furthermore, these results are also consistent with studies about surface wood application on reclamation soils in Northern Alberta that reported decreases in available nitrogen in FFMM and PMM [\(Brown and Naeth 2014;](#page-9-9) [Kwak et al. 2015](#page-9-10)*a*). The fFFMM is reported to have the highest nitrogen availability and a lower C:N ratio in comparison to other reclamation soils [\(McMillan et al. 2007;](#page-9-21) [Mackenzie and Quideau 2012;](#page-9-22) [Jamro et al. 2014\)](#page-9-23). Therefore, fFFMM had the greatest alteration on the C:N ratio and consequently the most noticeable nitrogen immobilization. PMM had a higher C:N ratio due to the greater proportion of organic matter and recalcitrant carbon [\(Hemstock et al. 2010\)](#page-9-24) and has been reported to have lower macronutrient content in comparison to fFFMM [\(McMillan et al. 2007;](#page-9-21) [Quideau et al. 2017\)](#page-10-9), which is majorly consistent with the findings in this study. cFFMM had a higher C:N ratio compared to fFFMM and PMM and the lowest nitrogen availability among all soils. Hence, the increase in the C:N ratio had no impact on nitrogen availability as this [soil already has an inherent low nitrogen content \(Wolkowski](#page-10-10) 1995; [Rees et al. 2020;](#page-10-11) [Wang et al. 2021\)](#page-10-12), but still, this increase was significant enough to impact the other macronutrients. Finally, peat had the highest initial C:N ratio and only responded to the highest additions of buried wood, indicating that this soil needs greater wood additions to have a significant change in the C:N ratio and subsequently impact nitrogen availability.

Phosphorus immobilization is more related to a higher P content than to an increase in the C:P ratio [\(Enwezor 1975;](#page-9-25) [Braakhekke et al. 1993;](#page-9-26) [Bünemann et al. 2012\)](#page-9-27). This is consistent with our findings since fFFMM, PMM, and peat already had a low P content; thus, there was no potential for immobilization. However, the decrease of available P in cFFMM suggests that there was an impact from buried wood addition and the increase in the soil C:P ratio. Buried wood had no impact on K supply rates in fFFMM, likely because K is immobile in soils with high clay content because it bonds strongly with the surface of the clay particles remaining fixed and unavailable [\(Lamp 1968;](#page-9-28) [Mengel et al. 1976;](#page-9-29) [Hoopen et al. 2010\)](#page-9-30). Additionally, fFFMM had high rates of magnesium and calcium that can outcompete K for exchangeable sites on the soil particles [\(Lamp 1968;](#page-9-28) [Reicks 2017\)](#page-10-13). Ammonium, Mg, and Ca have [been reported to interact and compete with K in soil \(Hoopen](#page-9-30) et al. 2010; [Bar Tal 2011\)](#page-9-31), which could explain why PMM, peat, and cFFMM had greater K availability as the buried wood increased, since the immobilization of these nutrients reduced competition.

Regarding the microbial communities, previous research has reported that wood addition and the consequent increment in the C:N ratio increase soil microbial respira[tion since wood decomposition is promoted \(Moritsuka et al.](#page-9-5) 2004; [Palviainen et al. 2010;](#page-9-19) [Mukhortova 2012;](#page-9-20) Truong and [Marschner 2018\), which explains the overall increase in mi](#page-10-8)crobial activity observed in all soils. Findings in the CLPP



showed how the microbial communities used carbon within a diverse set of metabolic pathways, supporting different anabolic and catabolic processes [\(Jones et al. 2018\)](#page-9-32). Buried wood changed the metabolic potential of the microbial communities in fFFMM and PMM, increasing the consumption of amino acids and carboxylic acids but not carbohydrates, implying that these two carbon sources were crucial to support the metabolic activity of wood decomposers in these soils. The high microbial activity in fFFMM is consistent with previous studies that report this soil has the greatest microbial activity among reclamation soils [\(McMillan et al. 2007;](#page-9-21) [Mackenzie and Quideau 2012;](#page-9-22) [Jamro et al. 2014\)](#page-9-23). Peat had a lower increase in microbial activity and metabolic potential likely due to the low pH and high organic carbon content of this soil. Acidic peat has been associated with lower microbial activity or  $CO<sub>2</sub>$  production when compared to peat with [neutral pH and reclamation soils like FFMM and PMM \(Ausec](#page-9-33) et al. 2009; [Naeth et al. 2013;](#page-9-34) [Mackenzie et al. 2014\)](#page-9-35), which also have a neutral pH. Previous studies have found that pH is the main factor predicting microbial activity and carbon [utilization patterns in peat and across soil types \(Fierer and](#page-9-36) Jackson 2006; [Preston et al. 2012\)](#page-10-14), as soil microorganisms have a narrow and sensitive pH range for optimal activity and growth [\(Rousk et al. 2010\)](#page-10-15). This explains why peat was the most distant soil in the ordination; considering it was the only soil with low pH, it had the most different microbial activity and metabolic potential. In addition, the high organic carbon content in peat makes carbohydrates the most abundant source of carbon, which explains why peat had the highest metabolic potential for carbohydrate consumption, along with the possibility of metabolic adaptation to the high abundance of this carbon source [\(Monod 1942;](#page-9-37) Táncsics et [al. 2020\). The cFFMM had no clear response to buried wood](#page-10-16) in terms of microbial metabolic potential, possibly because of the low nitrogen, organic matter content, and water holding capacity, crucial factors for supporting metabolic activity [\(Higashida et al. 1986;](#page-9-38) [Cao et al. 2016\)](#page-9-39).

# Implications for oil sands land reclamation practices

Buried wood addition can cause nitrogen immobilization due to an alteration in the soil C:N ratio and an increase in the demand for nitrogen by the soil microbial communities. Soils with a lower C:N ratio are more susceptible to nitrogen immobilization when buried wood is added to the soil. This means that when salvaging soils like fFFMM and PMM, it is recommended to monitor the amount of wood that is incorporated into the soil.

This study showed that fFFMM and PMM had a similar microbial activity, metabolic potential, and macronutrient supply, supporting the utilization of PMM in land reclamation as the mixing of peat and mineral material can result in a soil with similar characteristics of an upland soil, in this study the fFFMM.

However, the findings in this study are limited to PMM soils with a coarse mineral component (sand), since a PMM with a fine mineral component might have different responses. Although the cFFMM had lower nitrogen availability and microbial activity, this soil type might still be a suitable option for reclamation, as different reclamation objectives have different soil requirements, and coarse soils are suitable for the establishment and development of sites with drier moisture and poorer nutrient regimes.

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### Data availability

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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#### Competing interests

The authors declare there are no competing interests.

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